(data not shown). Similarly, blockade of EPCR on HUVECs with this EPCR blocking mAb did not have a significant effect on FX or FXa binding (Figure 1B). FVIIa and APC binding studies performed in parallel with FX or FXa experiments clearly demonstrated that both FVIIa and APC bound to cells to a similar degree and in an EPCR-specific manner (Figure 1A-B). Analysis of the binding of biotinylated, active-site blocked FXa, FVIIa and APC to EPCR on CHO-EPCR and HUVECs revealed that little FXa was bound to EPCR on cell surfaces compared with FVIIa or APC (Figure 1C-D). In this assay, FVIIa and APC both bound to EPCR with an apparent Kd of $\sim$ 15 to 25nM, whereas the Kd for FXa was $>$ 1μM. Consistent with these data that FX does not bind appreciably to EPCR, even a 100-fold molar excess of unlabeled FX (1μM) failed to compete effectively with the binding of ^{125}I-FVIIa (10nM) to CHO-EPCR cells (Figure 1E). Analysis of FX binding to EPCR expressing cells by confocal fluorescence microscopy did not show any detectable fluorescence, either at the cell surface or intracellularly, in CHO-EPCR cells or HUVECs exposed to FX tagged with a fluorescence dye (AF488; Figure 1F). In additional studies, we measured plasma levels of mouse factor X and protein C in EPCR overexpressing mice that received a high dose of active-site inhibited human APC. EPCR overexpression has been found to decrease circulating levels of protein C, while administration of human protein C has been shown to increase mouse protein C levels in circulation by displacing endogenous protein C from EPCR on the endothelium.[6] As expected from this study,[6] administration of human APCi increased plasma levels of mouse protein C (Figure 1G). In contrast to protein C, there was not a detectable increase in plasma levels of mouse FX after APCi administration (Figure 1H). These data indicate that FX does not effectively interact with EPCR in vivo, at least in regards to the mouse system. Overall our data indicate that FX binding to EPCR, if any, is minimal and likely physiologically insignificant. Our findings do not exclude the possibility that FX/FXa could indirectly interact with EPCR as suggested by others.[7]

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To the editor:

Linking air pollution exposure with thrombosis

We read with interest the review by Franchini and Mannucci examining the link between exposure to particulate matter air pollution (PM) and an increased tendency toward thrombosis.[1] In their discussion of the potential mechanisms by which PM might induce thrombosis, the investigators highlight the excellent work by Nemmar and colleagues suggesting that PM enhances the release of histamine by mast cells and the resulting activation of platelets increases the tendency toward thrombosis in hamsters administered intratracheal suspensions of diesel exhaust particles.[2]

We were surprised that the authors did not discuss an additional mechanism. In mice, we reported that the intratracheal administration of fine urban particulates or the inhalation of concentrated ambient particulate matter air pollution from Chicago resulted in an increase in the plasma levels of thrombin-antithrombin (TAT) complexes and accelerated arterial thrombosis in the ferric chloride carotid injury model via a mechanism that required the release of IL-6 from alveolar macrophages.[3,4] This mechanism is attractive as resident macrophages in the lung are likely the "first responders" to inhaled particles[5] and the prothrombotic effects of IL-6 have been

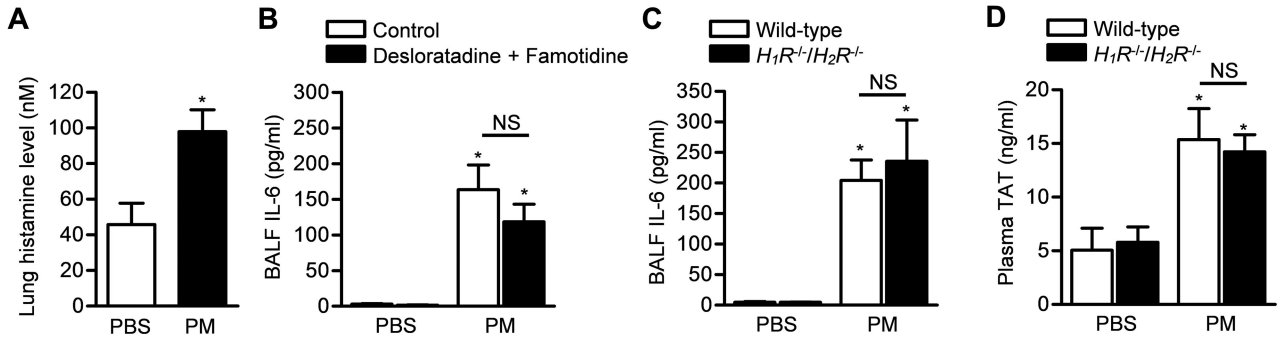

Figure 1. The effect of loss of histamine signaling in particulate matter–induced thrombin generation. Fine urban particulate matter (National Institute of Standards and Technology Standard Reference Material, SRM 1649a 200 μg/mouse in 50 μL PBS) or vehicle was administered intratracheally to 20-25 g, 6-8 week old male C57BL/6 mice as previously described.[4] (A) After 24 hours BAL fluid was obtained and histamine levels were measured using a commercially available assay (EIA Histamine IM2015, Beckman Coulter). (B) Mice were treated with famotidine (10 mg/kg) and desloratadine (10 mg/kg) in 150 μL of PBS 4 hours before treatment with PM followed by an additional dose of famotidine 8 hours later. Twenty-four hours after PM administration, BAL fluid was obtained and IL-6 levels were measured as previously described (ELISA).[3] (C,D) H1R and H2R receptor double knockout mice (*H1R*$^{-/-}$/*H2R*$^{-/-}$) or littermate controls were treated with PM and 24 hours later IL-6 and TAT were measured in BAL fluid and citrated plasma as previously described.[3] The protocol for the use of mice was approved by the Animal Care and Use Committee at Northwestern University. N = 4 or 5 animals for each group. *$P < .05$ compared with PBS control; and NS, not significant using ANOVA with Bonferroni posttest comparison.

well-described in the hematology literature.[6] The potential clinical importance of this mechanism is highlighted by the frequent observation in human populations exposed to particulate matter that IL-6 or its transcriptional target, C-reactive protein, are increased the day after the exposure.[7-9]

Like Nemmar and colleagues, we reported that the administration of particles resulted in an influx of macrophages and neutrophils into the lung.[3] However, we found that, while the instillation of PM increased histamine levels in the bronchoalveolar lavage (BAL) fluid (Figure 1A), the PM-induced increase in bronchoalveolar lavage fluid IL-6 was not affected by combined pretreatment of mice with pharmacologic inhibitors of histamine type I (H1R) and type 2 (H2R) receptors (Figure 1B). Furthermore, the PM-induced increase in BAL fluid IL-6 and the subsequent increase in plasma TAT complexes were similar in mice doubly deficient in the H1 and H2 receptors and their littermate controls (Figure 1C-D). As Franchini and Mannucci point out, much more work is required before we understand the important link between particulate matter air pollution exposure and thrombosis.

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To the editor:

Prediction of fetal status in fetal/neonatal alloimmune thrombocytopenia (FNAIT)?

We read with interest the recent paper from Bertrand et al,[1] which outlines an interesting topic regarding the use of antibody concentration as predictive parameter for FNAIT.

Unfortunately, the number of subjects studied is very low. When the authors conclude that FNAIT is a severe disease and the first offspring is already at high risk, they base their analysis on 66 "index cases." A significant difference in antibody concentration between primagravidae and multigravidae, however, is concluded from the analysis of 54% (20/37) and 34% (10/29) of enrolled cases only. Selection criteria are not given. In consequence, complete